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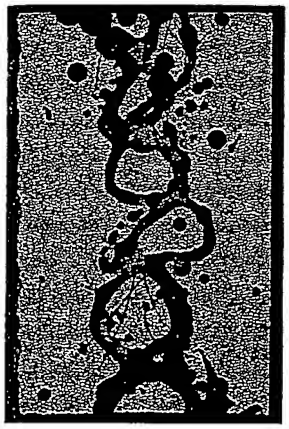

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Mutations in the gene encoding immunoglobulin μ-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1

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Classic spinal muscular atrophy (SMA) is caused by mutations in the telomeric copy of *SMN1*. Its product is involved in various cellular processes, including cytoplasmic assembly of spliceosomal small nuclear ribonucleoproteins, pre-mRNA processing and activation of transcription[1–8]. Spinal muscular atrophy with respiratory distress (SMARD) is clinically and genetically distinct from SMA[9–13]. Here we demonstrate that SMARD type 1 (SMARD1) results from mutations in the gene encoding immunoglobulin μ-binding protein 2 (*IGHMBP2*; on chromosome 11q13.2–q13.4). In six SMARD1 families, we detected three recessive missense mutations (exons 5, 11 and 12), two nonsense mutations (exons 2 and 5), one frameshift deletion (exon 5) and one splice donor-site mutation (intron 13). Mutations in mouse *Ighmbp2* (ref. 14) have been shown to be responsible for spinal muscular atrophy in the neuromuscular degeneration (*nmd*) mouse[15], whose phenotype resembles the SMARD1 phenotype. Like the *SMN1* product, IGHMBP2 colocalizes with the RNA-processing machinery in both the cytoplasm and the nucleus[16–19]. Our results show that *IGHMBP2* is the second gene found to be defective in spinal muscular atrophy, and indicate that IGHMBP2 and SMN share common functions important for motor neuron maintenance and integrity in mammals.

Autosomal recessive SMARD (also known as diaphragmatic spinal muscular atrophy[11,13], distal hereditary motor neuronopathy type VI, dHMN-VI (ref. 20) and severe infantile axonal neuropathy with respiratory failure[21]) and classic autosomal recessive SMA are both characterized by dysfunction and progressive loss of α-motor neurons in the anterior horn of the spinal cord, leading to neurogenic muscular atrophy with subsequent symmetrical muscle weakness of trunk and limbs[9–13]. In contrast to SMA, distal muscles are more severely affected in SMARD, and life-threatening respiratory distress with clinical and radiological evidence of unilateral or bilateral paralysis of the diaphragm is the most prominent presenting symptom[9–13] (Fig. 1 and Table 1). In previous studies, clinical and genetic heterogeneity of SMARD has been demonstrated. SMARD type 1 with non-congenital onset of respiratory distress has been linked to chromosome 11q13–q21 (SMARD1), whereas linkage to this locus could be excluded in one family with two affected children suffering from respiratory distress of congenital onset[12].

Symptoms similar to those of human SMARD1 have been found in *nmd* mice homozygous for autosomal recessive *Ighmbp2* mutations[14]. These animals suffer from progressive paralysis of the limbs with onset at 2 weeks of age, leading to death by 3.5 weeks secondary to respiratory failure[14,15]. Histopathological analysis showed progressive degeneration of α-motor neurons with secondary generalized atrophy of distal limb muscles[15].

We have refined the SMARD1 locus to a genetic interval of 9 cM with the centromeric critical breakpoint distal to *D11S913* and the telomeric breakpoint proximal to *D11S916* (data not shown). The most promising candidate gene within this region was *IGHMBP2*, as mutations of the mouse ortholog are responsible for the *nmd* phenotype[14]. Human *IGHMBP2* is composed of 15 exons (for exon–intron boundaries, see Web Table A). It is ubiquitously expressed[22], with the highest levels of *IGHMBP2* mRNA detected in testis and low-to-moderate expression in other human tissues[23].

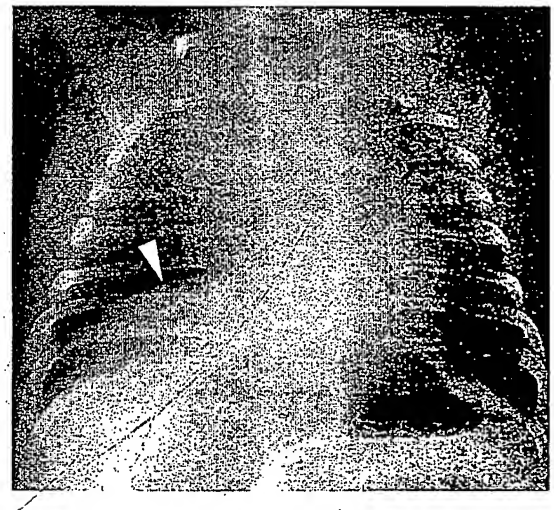

Fig. 1 Chest X-ray showing eventration of the right hemidiaphragm in a SMARD1 patient at 8 weeks of age. The infant presented with severe respiratory distress resulting from paralysis of the diaphragm.

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Table 1 • Mutations of *IGHMBP2* and clinical data in SMARD1 patients

Family	Affected/ unaffected sibs	Geographic origin	Consanguinity	Homozygous (heterozygous) mutation	Location	Amino acid substitution	Class of mutation	Age at onset of respiratory distress (weeks)
1	1/1	South Italy	no	1540G→A	exon 11	E514K	missense	4
2	5/3	Lebanon	yes	638A→G	exon 5	H213R	missense	6–9 (family 1 in ref. 12)
3	1/2	Turkey	yes	1738G→A	exon 12	V580I	missense	8
4	2/1	Germany	no	(121C→T)	exon 2	Q41X	nonsense	9–12
				(675delT)	exon 5	–	frameshift	(family 2 in ref. 12)
5	1/1	Lebanon	yes	707T→G	exon 5	L236X	nonsense	16 (patient 3 in ref. 21)
6	1/1	Sicily	yes	IVS13+1G→T	intron 13	–	splice donor	18

Family numbers correspond to those in Fig. 3.

DNA sequence analysis of four consanguineous families (families 2, 3, 5 and 6) and two non-consanguineous families (families 1 and 4) demonstrated seven different *IGHMBP2* mutations in four different exons and in one intron (Table 1). In family 1, a homozygous 1540G→A transition in exon 11 predicts substitution of a glutamic acid by lysine (E514K), and in family 2, a homozygous 638A→G transition in exon 5 predicts substitution of a histidine by arginine (H213R). This histidine residue is located within the first of seven helicase domains[24]. A homozygous 1738G→A transition in exon 12 (family 3) predicts replacement of valine by isoleucine (V580I). The residues E514, H213 and V580 affected by the three missense mutations are conserved between the orthologs of man, mouse, rat and golden hamster (Fig.2 ; Web Fig. A). In addition, we detected a heterozygous nonsense mutation in exon 2 (Q41X; family 4) and a homozygous nonsense mutation in exon 5 (L236X; family 5). In family 4, the second allele carried a 1-bp deletion in exon 5 (675delT), resulting in a nonsense peptide of six amino acids after V225, with subsequent chain termination. In family 6, a homozygous point mutation at the consensus splice donor site of intron 13 (IVS13+1G→T) probably results in defective splicing. Neither the splice donor-site mutation nor the missense mutations were detected in 50 unaffected unrelated individuals, indicating that these mutations do not reflect common polymorphisms. The *IGHMBP2* mutations segregate with the disease phenotype in all families (Fig. 3). This is consistent with the proposed autosomal recessive mode of inheritance[12] and supports the hypothesis that the mutations cause SMARD1.

We found a silent 180C→T sequence variation in exon 2 (Y60Y; family 2) and detected several variations in both affected people and healthy controls. These variations would seem to be polymorphisms (5′ untranslated region–136insGCCTCTTCCCGC, families 3 and 6; 2636C→A, T879K, families 2, 4 and 5; IVS14+54G→A, families 2 and 5). IGHMBP2 consists of 993 amino acids and includes 7 putative helicase motifs[24] and a DEAD box-like motif, which is typical for RNA helicases[17]. IGHMBP2 contains a DNA-binding domain at position 638–786 including the helicase motifs V and VI (refs. 19,22,24) and the nucleic acid-binding R3H motif[25]. The cellular function of IGHMBP2 is unknown. It is involved in

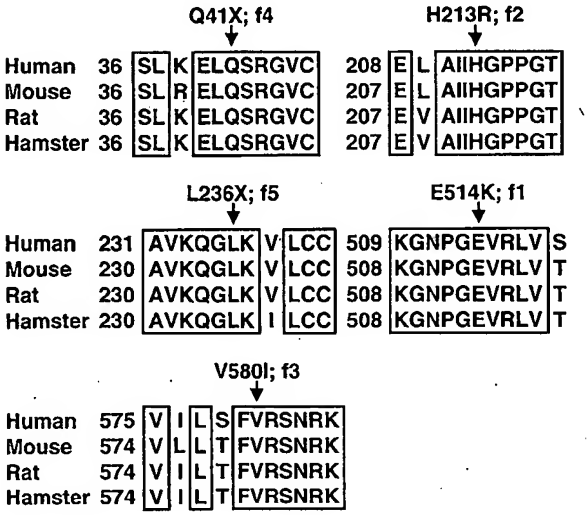

Fig. 2 Alignment of selected regions of human IGHMBP2 with orthologs of other species. Arrows indicate positions of the missense and nonsense mutations in SMARD1 patients. Family numbers correspond to those in Table 1. f1-5, families 1–5. (For alignment of the whole amino-acid sequences, see Web Fig. A).

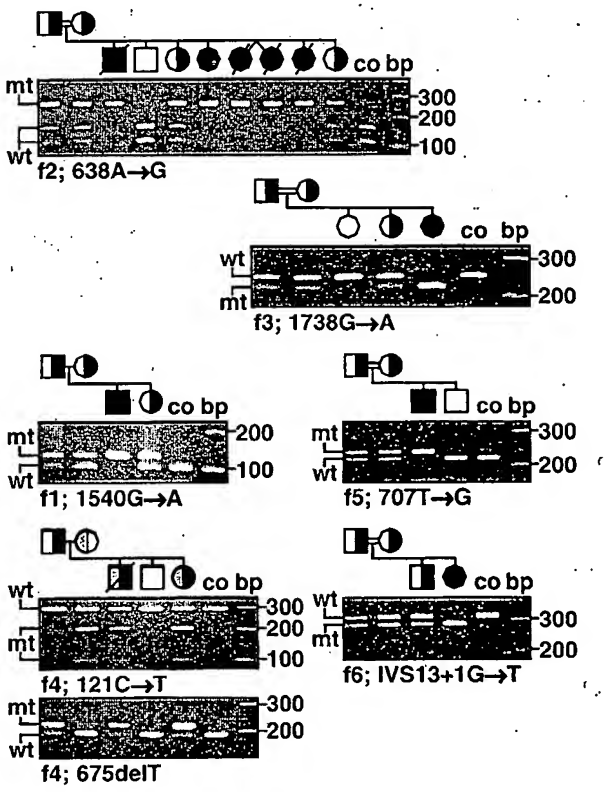

Fig. 3 Segregation of *IGHMBP2* mutations (restriction fragment-length polymorphism analysis). In family 4 (f4), the two affected siblings were compound heterozygotes carrying the maternal 121C→T nonsense mutation and the paternal 675delT frameshift deletion. Family numbers correspond to those in Table 1. co, control; f1-6, families 1–6; mt, mutated *IGHMBP2*; wt, wildtype *IGHMBP2*.

immunoglobulin-class switching[22], in pre-mRNA processing[17] and in regulation of transcription by DNA binding[16,19] or by interacting with TATA-binding protein[18]. In this respect, IGHMBP2 resembles the SMN protein, which is defective in classic SMA. SMN binds directly to DNA and RNA (ref. 26), activates transcription by association with the viral nuclear transcription activator E2 (ref. 4) and is involved in pre-mRNA processing[6,8]. Although SMN does not contain helicase motifs, it is part of a major cellular complex including DP103, a member of the DEAD box family of RNA helicases[3,5,27].

Our findings support the hypothesis that mutant SMN and mutant IGHMBP2 result in a similar dysfunction of spinal motor neurons, resulting in SMA and SMARD1, respectively. Functional characterization of IGHMBP2 will help to unravel the enigma of the cellular processes that underlie the specificity of diseases leading to neurogenic muscular atrophy.

Methods

Patients. We studied a total of 11 patients and 21 relatives from 6 unrelated families (Table 1). The diagnosis of SMARD1 was made on the basis of clinical criteria[11–13]. All affected infants were floppy, presented with life-threatening respiratory distress and had unilateral or bilateral eventration of the diaphragm on chest X-ray (Fig. 1). Surviving patients were on long-term artificial ventilation. In addition, analysis of muscle biopsy specimen showed neurogenic muscular atrophy. One patient (family 5) had bilateral equinovarus foot deformities at birth[21]. In all families, haplotype analysis was consistent with linkage to 11q13. We obtained blood samples from patients and family members after obtaining informed consent according to the declaration of Helsinki. We isolated DNA from peripheral blood lymphocytes, Guthrie card samples and skin fibroblast cultures according to standard procedures.

Haplotype analysis. We used 12 fluorescently labeled polymorphic markers and standard semi-automated methods[12] for microsatellite analysis. We used a MegaBACE 1000 DNA-sequencer and markers from the Généthon final linkage map: *D11S1883*, *D11S913*, *D11S4095*, *D11S4178*, *D11S1314*, *D11S916*, *D11S901*, *D11S1358*, *D11S1311*, *D11S4176*, *D11S1757* and *D11S917*.

Database analysis. The mRNA sequence of *IGHMBP2* has been published, and we used homology search and exon assembly with BLAST programs at the National Center for Biotechnology Information. We derived the genomic sequence of *IGHMBP2* from a contig of three genomic clones.

Sequence analysis. We amplified all 15 exons of *IGHMBP2* from patients' genomic DNA with intronic primers (see Web Table B). We used standard procedures for bi-directional automatic sequencing with fluorescent dye terminators on the MegaBACE 1000 DNA-sequencer with the above-mentioned primers.

We verified the intrafamilial segregation of the mutations by restriction-fragment-length polymorphism analysis (Fig. 3). When we found no natural restriction sites, we used primer-induced restriction analysis (primer mismatches underlined): 1540G→A, 11F/5′–CCTGGATGTGCAAACTGACGA GGCGGACGT–3′ (*Aat*II, mutated (mt)=124 bp, wildtype (wt)=98+26 bp); 638A→G, 5F/5R (*Nco*I, mt=255 bp, wt=149+105 bp); 1738G→A, 12F/5′–GGCTCCGTACCTTTCCTGTTGGATCTCA–3′ (*Bsp*HI, mt=211+30 bp, wt=241 bp); 121C→T, 2F/2R (*Xba*I; mt=92+199 bp, wt=291 bp); 675delT, 5F/5′–CCTTGTTTCACAGCTTGAAGAATGATGTCA–3′ (*Hin*cII, mt=216 bp, wt=188+29 bp); 707T→G, 5F/5′–TGGCATGCACTGCCCAC CCTT–3′ (*Afl*II, mt=241 bp, wt=211+30 bp); IVS13+1G→T, 13.2F2/5′–CACTGCCCCAAGTTCTTATTAGTTGAGTTA–3′ (*Hpa*I, mt= 277+29 bp, wt=306 bp).

Accession numbers. GenBank: *IGHMBP2*, L14754; *IGHMBP2* genomic clones, AP000808.2, AP000444.3, AC019166.5; *Ighmbp2* rat (*Rattus Norvegicus*), AF199411. OMIM: SMA1, #253300; SMARD1, *604320. SwissProt: IGHMBP2 human (*Homo Sapiens*), P38935; Ighmbp2 mouse (*Mus Musculus*), P40694; Ighmbp2 hamster (*Mesocricetus Auratus*), Q60560.

Note: Supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info/).

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